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Hypothesis

Enzymatic catalysis as a process controlled by protein conformational relaxation

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Great progress in studies of protein dynamics in the past decade propels an essential alteration in our understanding of the enzymatic catalysis phenomenon. A careful analysis of assumptions made by the hitherto used conventional theory of chemical reactions shows that neither of them is in fact satisfied. One of the reasons is the presence of a slow interconformational dynamics within the protein native state. In consequence, the simple classical statement "enzymes accelerate reactions by decreasing the free energy of activation" represents only half of the truth. Enzymatic reactions actually proceed through 'gates' of relatively low free energy but it is not the process of activated gate crossing that limits the reaction rate, but the process of generally non-activated gate opening, controlled by the conformational relaxation. Possible consequences of this fact are pointed out.

Reaction rate theory; Enzymatic catalysis; Protein dynamics; Conformational relaxation

1. INTRODUCTION

From the chemical point of view it is quite obvious at present that there is no mystery involved in the action of enzymes and that chemical mechanisms of enzymatic catalysis are identical to those of ordinary electrophilic/nucleophilic catalysis in the broad sense [1,2]. The physical picture is, however, not so clear because in macromolecular chemistry, contrary to the chemistry of low-molecular weight compounds, the *chemical* mechanism, pointing only to the way the covalent bonds are rearranged, does not have to relate directly to the *kinetic* mechanism, i.e. the decomposition of a complex reaction into elementary steps. In classical enzymology [1,2] both mechanisms are simply identified, with the latter completed only by reactions of enzyme–ligand binding and, if needed, by a rather hypothetical 'conformational change'. Thus, the reaction involving a single covalent step

$$R \leftrightarrow P \tag{1}$$

is modelled by the three-step kinetics of Haldane:

$$E + R \leftrightarrow ER \leftrightarrow EP \leftrightarrow E + P \tag{2}$$

or, having introduced two conformations of enzyme and its complexes, by the oscillating enzyme kinetics of Jencks:

$$\begin{array}{ccccccc} E & + R \leftrightarrow & ER & \leftrightarrow & EP & \leftrightarrow E & + P \\ \updownarrow & & \updownarrow & & \updownarrow & & \updownarrow \\ E' & + R \leftrightarrow & ER' & \leftrightarrow & EP' & \leftrightarrow E' & + P. \end{array} \tag{3}$$

The latter gives a formal meaning both to the idea of utilization of the free energy of enzyme–ligand binding (Haldane's *strain* or Koshland's *induced fit*) [1,2] and to the Volkenstein idea of electronic–conformational interaction, equivalent to the concept of *gating* [3].

The phenomenological kinetics alone does not explain, however, the catalytic action of enzymes. Because of the principle of detailed balance, each reaction path in Scheme (3) is favored to the same extent in the forward and backward direction and the most effective path reduces in fact to a linear Scheme like (2). Consequently, in order to elucidate enzymatic catalysis one has to go down to the microscopic level. Classical enzymology does it by resorting to the *transition state theory*. In its terms the catalytic action of enzymes consists simply in decreasing the activation free energy for the middle reaction of Scheme (2) when compared to that of the non-catalysed Reaction (1) and one can at most speculate about the importance of various enthalpic or entropic contributions [1,2].

The transition state theory and, more generally, any theory that treats chemical reactions as activated processes is based on the assumption that the equilibration among microstates within individual kinetic species is much faster than the very reaction [4]. In the present letter we point out that in the light of recent studies this

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assumption is by no means satisfied for proteins, which the enzymes represent. If so, we are compelled to change rather radically our understanding of the enzymatic catalysis.

The lack of interest of the classical approach in the internal dynamics of enzymes was often subject to criticism [5–7]. However, most attempts hitherto at formulating a dynamically oriented theory of enzymatic catalysis were far from being satisfactory. Neglecting some erroneus and not very clear concepts, the main reason for the failure of previous endeavours was their highly speculative character resulting from the lack of sufficient knowledge on the internal dynamics of proteins. Only now, due to essential progress in the studies in this field in the 1980s, one can attempt to approach a truly advanced statistical theory of enzymatic catalysis based on realistic models of phenomena underlying microscopic dynamics. We intend this letter to be a contribution proceeding in this direction.

2. MICROSCOPIC DYNAMICS OF PROTEINS

Let us point to the main features of the picture of protein dynamics emerging from the recent studies; more details can be found in author's short review [8].

The essential difference between protein macromolecules and small molecules considered by the ordinary physical chemistry is that the potential energy of their internal degrees of freedom has an astronomical number of local minima. As in the stereochemistry of small organic compounds we refer these minima to as *protein conformations* (note that in biochemical literature this term is rather poorely defined and used also in different meanings). In the first approximation [8], internal dynamics of proteins comprises *vibrations* within individual conformations and *conformational transitions*. The former are damped harmonic oscillations subjected to stochastic perturbations, of periods ranging from 10^{-14} s (localized N–H or C–H stretching modes) to 10^{-11} s (collective modes involving the whole domains), whereas the latter make purely stochastic activated processes with the spectrum of relaxation times beginning at 10^{-11} s (local side chain rotations or hydrogen bond rearrangements) and ending with the mean waiting-time of spontaneous protein's unfolding in the physiological conditions, estimated to be of 10^{5} s or longer.

Protein folding is a process of discontinuous phase-transition type, thus all the particular conformations in the physiological conditions are to be divided uniquely between the native (folded) and unfolded state. Until the end of the 1970s the native state of protein was commonly considered a single well-defined conformation and only Blumenfeld [5], Careri [9] and Frauenfelder [10] with a few allies advocated that it is in fact a dynamical mixture of a multitude of conformations. In the 1980s this view was confirmed with the help of various experimental techniques [8].

Conformational transitions within the protein native state take place not in the whole bulk of protein but are limited to liquid-like regions surrounding solid-like fragments of secondary structure surviving, in physiological conditions, the transition to the unfolded state. The spectrum of conformational relaxation times of native proteins is practically quasi-continuous. It is not simple in statistical physics to describe systems without a well-defined time scale separation. Two classes of models of proteins' conformational dynamics seem to be worth studying intensively [8]. In the first class, that could be referred symbolically to as *Protein-Glass*, the dynamics is assumed to be characterized by a hierarchy of interconformational barrier heights. In the second, *Protein-Machine* class of models, the variety of conformations composing the native state is supposed to be labelled with a few 'mechanical' variables, e.g. angles describing mutual orientation of rigid fragments of secondary structure or the whole domains.

3. INADEQUACY OF THE CONVENTIONAL THEORY OF CHEMICAL REACTIONS

In the contemporary non-equilibrium thermodynamics a distinction is made, if possible, between *slow* and *fast* dynamic variables. Thermodynamic non-equilibrium is considered as a *partial equilibrium* achieved by fast variables for instantaneous values of slow variables. In the conventional theory of chemical reactions, underlying the classical approach to enzymatic catalysis, the only postulated slow variables are *concentrations* of kinetically distinguishable species. It needs the following assumptions to be satisfied [4].

Assumption 1: statistical independence of molecules (or pairs of molecules reacting at a given time). This assumption limits theoretical considerations to microstates of a single molecule or a pair of molecules and is equivalent to the mean-field approximation commonly made in many branches of condensed phase physics.

Assumption 2: activated mechanism of reactions. According to this assumption, as a consequence of the presence of potential energy barriers on the borders between subsets of microstates belonging to individual species, the processes of internal equilibration of microstates within individual species are much faster than the process of achieving equilibrium between the species.

In the transition state theory the internal equilibration of microstates is assumed to be infinitely fast, also at the borders between the subsets. Such a sharp formulation of Assumption 2 is, however, not necessary for the equations of conventional chemical kinetics to be valid arbitrarily far from equilibrium [4]. In fact, the assumption of infinitely fast internal equilibration is certainly not valid for any macromolecular reaction, simply because the lifetime of the transition state (of the

order of 10^{-13} s at room temperature) is much shorter than the period of low-frequency collective modes of vibrations, not ocurring in small molecules.

It is not the intention of the present letter to be yet another criticism of the application of transition state theory from the point of view of the slowness of *vibrational* relaxation. Our objection to the classical description of enzymatic catalysis is much more serious because of the existence of a *conformational* relaxation being as slow, if not slower, as the very enzymatic reaction of typical rate one per 10^{-3} s (compare Fig. 1 of [8]). Certainly, some conformational transitions are independent of the reaction but others must affect the latter in a way. In consequence, the component steps in Scheme (2) cannot be generally considered as activated processes. To describe the actual kinetic mechanism of enzymatic reaction one has to treat all conformational (non-covalent) transitions on an equal footing with the chemical transformations. The resultant scheme for the enzymatic reaction involving a single covalent step is infinitely more complex than Eqn. (3):

$$\begin{array}{ccccc} E_i + R & \leftrightarrow & ER_i & \leftrightarrow & EP_i \leftrightarrow E_i + P \\ \updownarrow & & \updownarrow & & \updownarrow \quad \updownarrow \\ E_j + R & \leftrightarrow & ER_j & \leftrightarrow & EP_j \leftrightarrow E_j + P \end{array} \qquad (4)$$

with indices i and j running over a quasi-continuous set of conformations of the enzyme or its complexes.

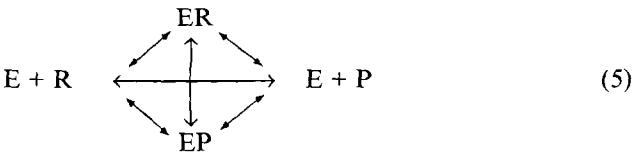
The validity of Assumption 1 for biochemical reactions should also be commented on. At present, there is strong evidence that single enzyme molecules occur as statistically independent objects practically only in vitro. In vivo, many enzymes belonging to fundamental metabolic cycles (including, probably, also membrane phosphorylation) occur in the form of supramolecular multienzyme complexes [11]. These should be considered the universal statistically independent units of biochemical processes. Intermediate metabolites are channeled to internal microcompartments of such complexes so the corresponding concentrations are microscopic rather than thermodynamic variables. In consequence, it is not correct to study a single chemical Reaction (1) independently of other reactions in a metabolic pathway, and the Scheme (3) should be extended also horizontally. Because of the limited space we shall, however, not continue discussing this topic here.

4. POSSIBLE CONSEQUENCES

It is obvious that, on the phenomenological level, a scheme like (4) is operationally useless as one cannot distinguish (and prepare) so many species, and no detailed investigation of the reaction time course can verify it. As in the classical approach, the process should be described phenomenologically in terms of kinetic equations for a few *observable* concentrations: [E], [ER] and [EP], and the only, but serious consequence of a 'dynamical disorder' within particular species is that the rate parameters involved are formally random functions of time [12]. The problem of formulating reasonable approximations of those equations is highly nontrivial and, at present, only partial solutions are available [13,14].

In general, the time course of reaction can be divided into two stages. The first stage, dependent on the initial distribution of conformations in the ensemble of enzyme macromolecules, begins for this distribution remaining practically frozen (a 'static disorder' [12]) and ends with this distribution partially equilibrated for given values of dynamic variables controlling the reaction. The latter relax to the total equilibrum in the second stage of the reaction. In the Protein-Glass model the slow variables are concentrations of 'taxonomic' conformations, making the lowest level of the hierarchy of conformational states [10,13], whereas in the Protein-Machine model these are average values of mechanical variables determining relative orientation of subunits composing the enzyme macromolecule [14]. Only for special conditions [14] the slow variables can be the concentrations of chemical species, i.e. the reaction is an activated process in the sense of Assumption 2.

The crossing-over between the two stages of the reaction moves to shorter time periods when the temperature rises [13,14] so the first stage may appear to be directly observable only in the low temperatures. This does not, however, mean that in physiological temperatures the initial stage microscopic dynamics does not play any role. If there are several chemical species distinguished, like in Scheme (2), the conformational nonequilibrium causes the reaction to proceed between each pair of species, with equilibration in intermediates omitted. Consequently, the effective kinetic scheme should look like



(5)

rather then (2). Scheme (5), if extended to include a few metabolic reactions proceeding on the same multienzyme complex, describes *direct* coupling of reactions, a mechanism to be distinguished from *thermodynamic* coupling considered in the classical biochemistry. The importance of the novel mechanism cannot be overestimated and it is worth considering in a separate paper.

Not less important is the fact that the initial stage conformational relaxation characterizes behaviour of biomolecules in the steady-state conditions far from equilibrium, under which most biochemical processes take place. It is this initial conformational relaxation, and not the effective second-stage rate constants for Schemes (2) or even (5), that determines the value of the turnover number, the basic phenomenological parame-

ter of enzymology. This idea is due to the late L.A. Blumenfeld [5], but only now the time has come to formalize it and put to the verification.

5. CONCLUDING REMARKS

Great progress in the studies of protein dynamics in the past decade propels an essential alteration in our understanding of the enzymatic catalysis phenomenon. The simple classical statement '*enzymes accelerate reactions by decreasing the free energy of activation*' represents only half of the truth. We do not deny enzymatic reactions to proceed through 'gates' of relatively low free energy, e.g. transient packing defects giving free space for substrate motions, or local polarization fluctuations facilitating displacement of charges. Gates are characterized by special position of several groups of atoms at the same time and most enzymologists considering details of such configurations do not worry about how they are reached, simply assuming that it can be done by an equilibrium fluctuation – the 'transition state'. But we argued that relaxation towards the equilibrium is very slow so *it is not the process of activated gate crossing that limits the reaction rate, but the process of, generally non-activated, gate opening.*

New approach changes the interpretations but leaves the phenomenology essentially unaltered. The steady-state kinetics of single enzymatic reaction is, under not very restrictive conditions, still to be described by the Michaelis-Menten law [1,2] and the transient time course can be always fitted with a few exponentials. Also Arrhenius temperature dependence of the effective rate parameters realizes in a wide class of models. Consequently, it is not simple to carry out experimentum crucis falsifying directly the classical interpretation. We suppose that besides studies in low temperatures [10,13] important could be a careful demonstration of the lack of direct relation between the value of the turnover number and the values of effective rate constants near equilibrium.

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